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Specific nongenomic actions of aldosterone

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Background. According to the traditional model, steroid hormones modulate gene transcription and protein synthesis. The considerable latency of these genomic steroid effects is the consequence of these time-consuming steps of action. Over the years, it has become increasingly clear that rapid actions of steroids exist that are incompatible with this "classic" genomic model of action. These rapid, nongenomic effects, which recently have been shown for virtually all groups of steroids, are likely to be transmitted by specific membrane receptors.

Methods. A review of data mainly focusing on the nongenomic in vitro and in vivo effects of aldosterone is presented.

Results. For rapid aldosterone effects, a prominent example of a receptor/effector cascade for nongenomic steroid effects has been described in various cell types. Nonclassic membrane receptors with a high affinity for aldosterone, but not for cortisol, seem to be involved. As an important second messenger, $[Ca^{2+}]_i$ is consistently increased within minutes after the addition of aldosterone. The effects are half maximal at physiological concentrations of free aldosterone (approximately 0.1 nmol/L), while the classic mineralocorticoid antagonist canrenone is ineffective in blocking the action of aldosterone. In addition, cortisol is active only at supramicromolar concentrations. Aldosterone rapidly acts on further cell signaling systems, for example, phosphoinositide hydrolysis and cAMP generation.

Conclusions. For a better understanding of nongenomic aldosterone action even in a clinical context, future research will have to target the cloning of the first membrane receptor for aldosterone and the evaluation of the clinical relevance of rapid steroid effects in general.

Traditionally, steroid hormone action has been described as the modulation of nuclear transcription, thus triggering genomic events that are responsible for physiological effects [1]. Steroid hormones influence gene transcription via interaction with intracellular receptors that are considered to represent a protein superfamily because of homologies in their molecular structure [2]. Being sensitive to inhibitors of transcription and translation, for example, actinomycin D and cycloheximide, related and delayed physiological responses are classified as genomic actions of steroids.

During the past two decades, it has been shown that steroid hormones can elicit rapid, nongenomic effects that are incompatible with genomic mechanisms of steroid action. Nongenomic steroid action is mainly characterized by its rapid onset and its insensitivity toward actinomycin D and cycloheximide. Evidence for nongenomic steroid effects has been provided for all classes of steroid hormones, including the secosteroid vitamin D_3 and triiodothyronine [3, 4]. In the context of nongenomic steroid action, specific binding sites for various steroids have been described in cellular membranes, exposing pharmacological properties distinct from those of the classic intracellular receptors [3]. Examples of rapid nongenomic steroid action include the rapid stimulation of ion fluxes and the acrosome reaction in human sperm by progesterone [5, 6], as well as the modulation of Ca^{2+} fluxes induced by testosterone [7, 8] and vitamin D_3 [9]. The rapid, nongenomic actions of the mineralocorticoid aldosterone are specified in this article.

HISTORY

The first data on the rapid nongenomic effects of aldosterone were reported about 35 years ago. Spach and Streeten demonstrated in vitro effects of aldosterone on sodium exchange in dog erythrocytes at physiological aldosterone concentrations [10]. Because of the lack of a nucleus, in vitro effects in these cells cannot be genomic in nature and therefore must be nongenomic ones. The first acute cardiovascular effects of aldosterone in humans were reported by Klein and Henk [11]. Increased peripheral vascular resistance and blood pressure and decreased cardiac output were measured within five minutes after the administration of aldosterone (0.5 mg intravenously), suggesting a nongenomic mechanism of action because of the short time frame. Despite these early observations, research on the mechanisms for nongenomic actions of aldosterone is relatively recent [12, 13].

IN VITRO EFFECTS OF ALDOSTERONE

Further investigations on rapid aldosterone action, triggered by observations on [^{3}H]aldosterone binding to lymphocytes [14], were done with regard to electrolyte

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transport, including changes of intracellular electrolytes, cell volume, and an activation of the sodium-proton exchanger of the cell membrane. These studies on time course, pharmacology, and the membrane receptors and second messengers potentially involved were done in human mononuclear leukocytes (HMLs), vascular smooth muscle cells (VSMCs), and porcine aortic endothelial cells (PAECs).

In HMLs, aldosterone significantly increased intracellular Ca^{2+} ($[Ca^{2+}]_i$), potassium, sodium, and the cell volume within one hour, thus in a time range in which genomic actions cannot be ruled out. Half-maximal effects were demonstrated at approximately 0.1 nmol/L aldosterone [13, 15, 16]. However, very rapid aldosterone effects have also been shown in these cells. For example, an aldosterone-induced activation of the sodium-proton exchanger was determined, which was significant after only one to two minutes of incubation. The EC_{50} of this effect was also in the subnanomolar range. Cortisol and other glucocorticoids were active at supramicromolar concentrations only, suggesting the involvement of a novel receptor, as the classic intracellular aldosterone receptor binds mineralocorticoids and glucocorticoids with similar affinities. Moreover, the type I mineralocorticoid receptor antagonist canrenone as well as actinomycin D and cycloheximide did not block these effects [17].

In VSMCs, a similar effect of aldosterone on the sodium proton exchanger was found with an apparent EC_{50} of about 0.2 nmol/L. Similar to the studies in HMLs, cortisol was ineffective at micromolar concentrations, and canrenone did not block aldosterone effects at 1000-fold excess concentrations [18].

In further studies, nongenomic aldosterone action on cell signaling systems with respect to phosphoinositide hydrolysis [production of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG)] was investigated in both HMLs and VSMCs. In these cells, aldosterone, as well as the synthetic mineralocorticoid fludrocortisone, significantly stimulated the generation of IP_3 within 30 seconds at an EC_{50} of approximately 0.1 nmol/L. Dexamethasone and cortisol were weak agonists, showing EC_{50} values in the supramicromolar range. As demonstrated for activation of the sodium-proton exchanger, canrenone did not antagonize these effects at 100-fold excess concentrations [18, 19].

Like IP_3, DAG production was increased in VSMCs within 30 seconds by subnanomolar concentrations of aldosterone, but only by supramicromolar concentrations of cortisol [20].

As IP_3 releases Ca^{2+} from intracellular IP_3-sensitive stores [21], the effects of aldosterone on free $[Ca^{2+}]_i$ were investigated in VSMCs and PAECs by single-cell, dual-wavelength fluorometry and cell imaging employing Fura2 fluorescence [22, 23]. After the addition of aldosterone, an immediate increase of Ca^{2+} was seen, which reached a plateau within two to three minutes. Consistent with the results described previously in this article, in VSMCs, the EC_{50} for aldosterone was approximately 0.1 nmol/L, while cortisol and other glucocorticoids were active at or above micromolar concentrations only. The aldosterone antagonist spironolactone (10 μmol/L) did not antagonize the effect of aldosterone [22, 23]. These results are in line with studies by Doolan and Harvey, who investigated rapid aldosterone-induced effects on $[Ca^{2+}]_i$ of rat distal colon [24].

Since the mentioned EC_{50} of approximately 0.1 nmol/L is similar to the concentration of free aldosterone in human serum [25], a physiological role of the rapid aldosterone-induced effects may be hypothesized, as they neither require concentrations, which are too high to occur under physiological conditions, nor would they be expected to be completely active under these conditions.

Depletion of $[Ca^{2+}]_i$ stores by thapsigargin blocked the aldosterone-dependent release of Ca^{2+}. In Ca^{2+}-free medium, the initial rise of Ca^{2+} seen after aldosterone stimulation was still visible, whereas the plateau had vanished. Apparently, this nongenomic steroid effect involves an initial release of Ca^{2+} from intracellular stores, followed by an influx of extracellular Ca^{2+}. However, between various cell types, both contributions seem to differ in their relative significance. The release of Ca^{2+} from intracellular stores was found to be the predominant effect in VSMCs. In PAECs, Ca^{2+} influx is more prominent, as shown by single-cell imaging and the more pronounced blunting effect of removal of the external Ca^{2+} [22]. In addition, in latter cells, the EC_{50} value of aldosterone is lower than in VSMCs by two orders of magnitude [26].

In contrast to the Ca^{2+} effects induced by peptide agonists such as thrombin or angiotensin II, rapid nongenomic aldosterone action on $[Ca^{2+}]_i$ is rather small in VSMCs and PAECs. Therefore, this mechanism appears to be the first known "low ceiling" effector with an inherent safety margin, and may be useful as an instrument to fine tune cardiovascular reactions. This potential role in cardiovascular regulation is further supported by data on Ca^{2+} increase in VSMCs induced by angiotensin II, which, after a short preincubation with aldosterone, is active at 100-fold lower concentrations [23].

Inhibitors of tyrosine kinase and phospholipase C block rapid aldosterone effects on free $[Ca^{2+}]_i$. While short-term treatment with phorbol esters blocked aldosterone effects on $[Ca^{2+}]_i$, the protein kinase C inhibitor staursporine increased these effects, indicating an involvement of protein kinase C in rapid aldosterone signaling. In VSMCs, the nongenomic effects of aldosterone on protein kinase C α have also been directly demonstrated by immunostaining. Aldosterone rapidly induces a translocation of protein kinase C α isoform from

cytosol to plasma membrane [20], an effect that is considered to be correlated with protein kinase C activation.

Triggered by the data on rapid aldosterone action on $[Ca^{2+}]_i$ and phosphoinositide hydrolysis in VSMCs, the nongenomic effects of the steroid on intracellular cAMP levels were recently investigated [27]. In porcine coronary VSMCs (PCVSMCs), an aldosterone-induced increase of intracellular cAMP levels by approximately 1.5- to 2.5-fold was measured within one minute. The EC_{50} of this effects was approximately 0.01 to 0.1 nmol/L. A 15-minute preincubation of cells with spironolactone (10 μmol/L) did not significantly blunt this effect. This effect was not blocked by inhibitors of transcription or protein synthesis and was found to be Ca^{2+} dependent. 17β-estradiol and hydrocortisone act at only supraphysiological concentrations (10 μmol/L).

Moreover, aldosterone-induced phosphorylation of CRE binding protein (CREB) was investigated because it may link cAMP levels to transcription. Stimulation of PCVSMCs with aldosterone increased immunodetectable phosphorylation of CREB within minutes, whereas the addition of the solvent alone did not significantly influence CREB phosphorylation [27].

MEMBRANE BINDING SITES FOR ALDOSTERONE

The rapid time course, along with the unique pharmacological properties of the nongenomic action of aldosterone and other steroids, is not covered by the classic genomic concept of steroid action. Therefore, nongenomic steroid effects are supposed to act via different pathways, including receptors distinctly different from those involved in genomic steroid actions.

Steroid membrane-binding sites have been described and partly characterized in various tissues and are thought to be receptor candidates for rapid steroid signaling [3]. In particular, binding sites for aldosterone have been found in HMLs, pig liver, and kidney by the use of radioactively labeled aldosterone analogues [28–30].

In binding studies with microsomes of porcine kidney and HMLs, specific saturable binding was demonstrated at a kD of approximately 0.1 nmol/L for the radioligand; displacement experiments showed a kD of approximately 0.1 nmol/L for aldosterone [28, 30]. Canrenone and cortisol were inactive as ligands up to micromolar concentrations, whereas fludrocortisone and desoxycorticosterone acetate had an intermediate activity.

As aldosterone-binding characteristics, with regard to important kinetic and pharmacological properties, are in perfect agreement with the functional data of rapid aldosterone effects mentioned previously in this article, these binding sites therefore may mediate rapid aldosterone action. These results are also in line with those reported for cultured kidney cells [31] and distal rat colon [32]. The data on rapid aldosterone action and potentially related binding sites are incompatible with an involvement of classic intracellular type I mineralocorticoid receptors, which do not discriminate aldosterone and cortisol and bind canrenone with a similar affinity.

To characterize these aldosterone membrane binding sites further, microsomal preparations from porcine liver membranes were used that showed a maximum binding capacity of approximately 700 fmol mg^{-1} microsomal protein [29]. Binding of [^{3}H]aldosterone was saturable, and Scatchard analysis revealed two apparent dissociation constants of <11 nmol/L and 118 nmol/L, respectively. A further purification of the aldosterone binding sites was unsuccessful because of the instability of the protein in its solubilized form. However, a progesterone-membrane binding protein from porcine liver microsomes has been characterized and cloned, which is likely to represent the first steroid membrane receptor, or a part of it [33, 34].

IN VIVO EFFECTS OF ALDOSTERONE

In recent years, there has been a significant increase of reports on the rapid in vivo actions of steroids. Particularly, the rapid nongenomic effects of estradiol on vasoregulation have gained extensive interest because of their possible clinical relevance in hormone replacement therapy during menopause.

Convincing in vivo evidence has been described for rapid aldosterone action, including effects on the baroreceptor neuron discharge frequency in the dog, which occur as early as 15 minutes after the application of the steroid [35]. In addition, aldosterone was found to significantly increase peripheral vascular resistance and blood pressure in humans, while cardiac output was decreased within five minutes after an injection of aldosterone [11]. The results of the latter study have been confirmed in catheterization studies by using modern invasive techniques. Systemic vascular resistance was significantly increased after an intravenous application of 0.5 mg aldosterone within three minutes [36]. Further clinical significance for rapid aldosterone action was found in a study in which calf phosphocreatine concentrations were monitored by nuclear magnetic resonance spectroscopy at rest and under stress [37]. Aldosterone (0.5 mg intravenously) significantly facilitated phosphocreatine recovery after stress, an effect starting within eight minutes after the application of the steroid.

INTEGRATIVE MODEL OF ALDOSTERONE ACTION

Taking all of the findings demonstrated previously in this article together, it becomes clear that the classic

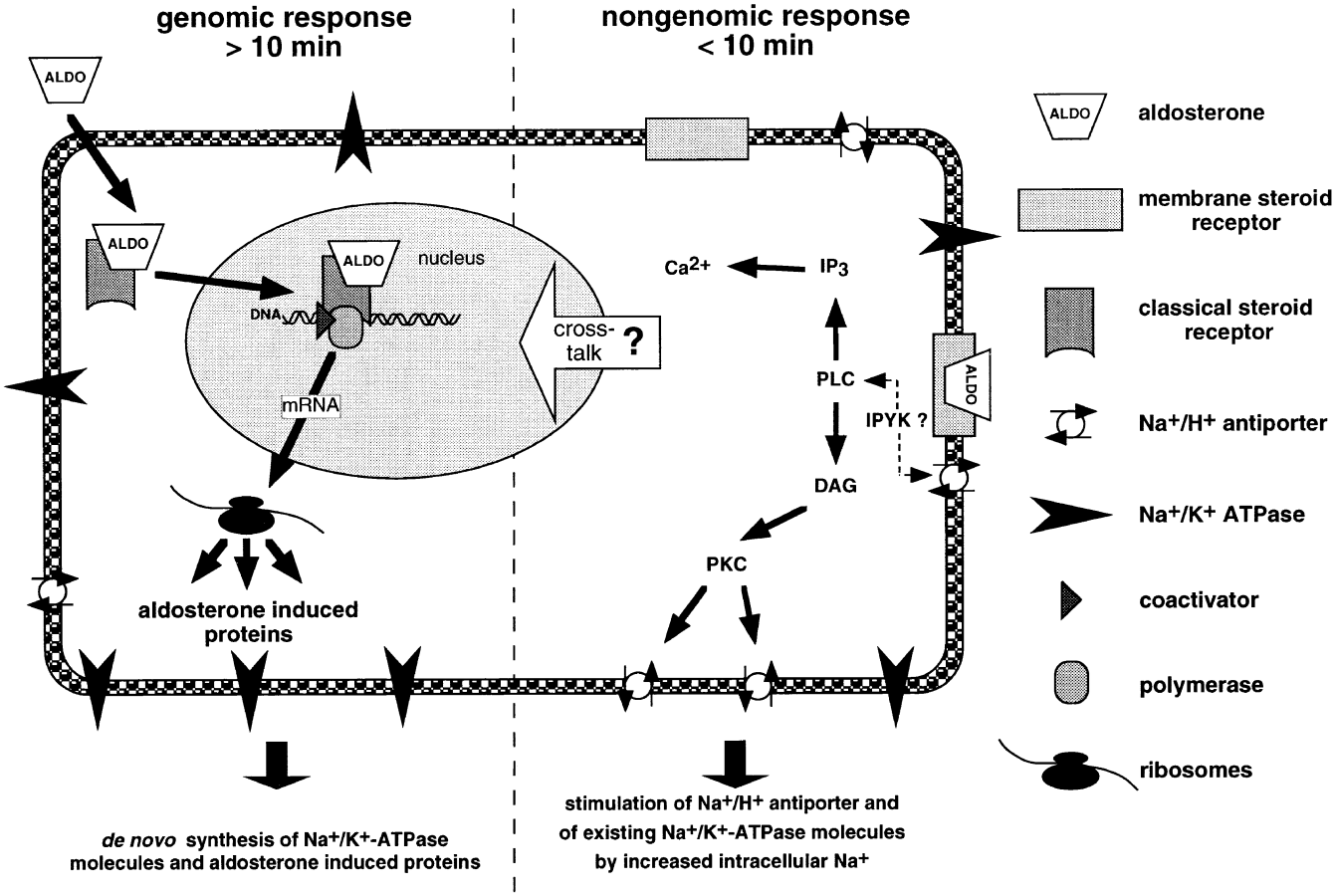

Fig. 1. The integrative two-step model for mineralocorticoid action. In addition to the scheme representing the classic genomic theory of mineralocorticoid action (left), the rapid pathway is shown (right), summarizing observations on rapid effects of aldosterone on the sodium-proton exchanger (Na^+/H^+ antiporter), intracellular second messengers (IPYK, intermediate tyrosine kinase; IP_3, inositol-1,4,5-trisphosphate; DAG, diacylglycerol; $[Ca^{2+}]_i$, free intracellular calcium; PKC, protein kinase C), and aldosterone binding to membrane receptors (reprinted from Christ and Wehling [38], used with permission of *Cardiovasc Res*).

genomic model does not adequately cover all aspects of steroid action. Therefore, a two-step model of steroid action has been recently proposed (Fig. 1) [3, 38]. Although originally developed for mineralocorticoids, it appears to be applicable to most steroids. On the one hand, the model describes nongenomic steroid action that comprises binding to membrane receptors followed by cellular signaling. On the other hand, genomic steroid actions are enclosed that involve steroid binding to intracellular receptors, resulting in the modulation of transcription. Moreover, as a yet unproved hypothesis, the interaction of both pathways is included in the model because there is increasing evidence for second messenger-related modulation of steroid-induced transcriptional processes [39, 40]. Steroid hormones may act through both mechanisms simultaneously, and these mechanisms appear to be important co-mediators in the wide range of cellular steroid effects.

CONCLUSION

In addition to the multitude of nongenomic aldosterone effects described during the past two decades, the identification, characterization, and cloning of the first aldosterone membrane receptor remain the major goals. Thereafter, researchers will be able to efficiently generate specific agonists and antagonists for the further characterization of the relevance of nongenomic steroid action with regard to physiology and pathophysiology. Development of new therapeutic compounds that are able to inhibit rapid steroid actions, or even both the nongenomic and genomic effects, could yield therapeutic benefits in a variety of clinical fields, including cardiovascular, obstetric, and endocrinologic disorders.

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